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Laboratory evaluation of an improved electronic grain probe insect counter

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Abstract

An Electronic Grain Probe Insect Counter system, which incorporates modified passive grain probes, allows offsite monitoring and detection of insect pests in stored grain. An electronic count is generated whenever an insect falls through an infrared beam in the sensor head located at the bottom of the electronic grain probe. We report descriptions and laboratory evaluations of prototype electronic grain probes that were custom-made in-house (n = 8) and by small-scale manufacturing (n = 54). Laboratory tests, in which dead insects were dropped through a probe, were conducted to determine if electronic probes accurately count the numbers of insects that are captured. Accuracy of the manufactured electronic probes increased as the size of the test insect increased from 93.6% for the smallest insect tested (Cryptolestes ferrugineus, the rusty grain beetle) to 99.5% for the largest (Tribolium castaneum, the red flour beetle). Custom-made probes were significantly more accurate for C. ferrugineus (96.5% versus 93.6%) but there was no difference in accuracies for the larger insects. Comparisons among all probes found that probe accuracy was correlated with variation in the magnitude of the output signal from the infrared phototransistor. Thus, use of diode/phototransistor pairs with a more consistent beam or with improved beam focus may further improve probe accuracy. Good performance was obtained with the manufactured electronic probes. Tests with live insects under field conditions are needed to further evaluate the system performance. Published by Elsevier Science Ltd.

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1. Introduction

Pitfall grain probes are commercially available traps that are used for detecting adult insect populations in stored grain (White et al., 1990). Information from these traps can also be used to estimate insect populations (Lippert and Hagstrum, 1987; Hagstrum et al., 1991, 1998). Traps are pushed into the grain and insects moving through the grain enter the holes in the perforated trap body, fall through and are captured in a collection receptacle. Traps must be removed from the grain bin and inspected periodically to determine the number of insects that have been captured. This is labor intensive, limits the temporal availability of data, and restricts placement of the probe traps to easily accessible locations. The Electronic Grain Probe Insect Counter (EGPIC), which consists of multiple probe trap bodies, each with an infrared-beam sensor head attached to its bottom, beam generation/detection circuitry, and a computer interface, has been developed to overcome these limitations (Shuman et al., 1996; Litzkow et al., 1997). An electronic count is generated whenever an insect that has crawled into a probe body falls through the sensor head. The prototype version of the system employs a detection/computer interface circuit box that connects to eight probes and conditions their sensors' output signals into a form suitable for transmission to a dedicated computer via its parallel (printer) port. A complete EGPIC computer program provides many features such as a menu-driven user interface, a data acquisition screen that displays cumulative capture per probe, and creation of a file with a time-stamp for each insect count.

Within each sensor head is a transverse light beam projected by a light-emitting diode (LED) and received by a phototransistor. Infrared transducers were used to avoid interference by ambient light and were selected for their narrow beam divergence. The range of sizes for the stored-product insect species of concern (from $1.5 \text{ mm} \times 0.4 \text{ mm}$ for Cryptolestes pusillus (Schönherr), the flat grain beetle; to $4.5 \text{ mm} \times 1.1 \text{ mm}$ for Tribolium castaneum (Herbst), the red flour beetle) is smaller than the transducers' diameter (5 mm) and so the passage of an insect through the head only partially masks the beam. Also, the phototransistor is biased in its linear region to produce an output signal proportional to the decrease in irradiance. This results in output signals that vary with insect size and allows the minimum detectable object size to be set by means of an adjustable threshold voltage.

The first generation prototype electronic probes used the trap body and receptacle of a Storgard WB Probe II probe trap (Trécé, Salinas, CA) for the upper and lower body sections, and a sensor head, fabricated by molding resin and by machining PVC stock, that was sandwiched between the two sections. Although there was good performance in initial laboratory tests (Shuman et al., 1996), some species (e.g., *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, and *Sitophilus oryzae* (L.), the rice weevil) could grasp the infrared-beam transducers and produce multiple counts (Shuman and Coffelt, 1994). This was remedied by the addition of a clear acetate insert partially coated with Teflon film. A field test of this first generation system, conducted in a flat storage of corn in WI (D.S., unpublished data), revealed some deficiencies in the sensor probe design that affected the ability to obtain the actual insect count. For example, grain particles entered through the holes in the probe body and were counted as they fell through the infrared beam. There was also a buildup of grain dust on the clear insert, which masked the beam and provided a foothold for insects.

The first generation sensor probes were modified to improve accuracy and dependability of the system, and a second generation sensor probe was developed (Shuman et al., 2000). Results of field trials conducted in FL with the second generation prototype probes (Arbogast et al., 2000) were encouraging and there was an interest in expanding the field tests of the EGPIC system. The limiting factor in this expansion was the small number of EGPIC systems available. All of the components for the sensor head of the second generation prototype probes had been produced in-house at the USDA/ARS laboratory in Gainesville, FL. There are several labor-intensive steps needed to produce these components, but some are amenable to commercial manufacturing processes. The sensor probes are the critical components in determining the system's performance. Ability to precision mill the sensor head components using commercial manufacturing processes has allowed small-scale replication of the second generation prototype EGPIC system.

We report herein descriptions and laboratory evaluations of the second generation prototype electronic probes that were produced in-house and by small-scale manufacturing. Tests were conducted to determine if the materials and manufacturing procedures that were used would produce electronic probes that accurately counted the number of insects that were captured. Availability of a number of manufactured EGPIC system components also allowed assessment of the factors that affect the accuracy of the electronic probes.

2. Materials and methods

2.1. Design of second generation sensor probe

To reduce the number of grain particles entering the probes, the Grain Probe Insect Trap (Thermo Trilogy, Columbia, MD), which is an acrylic tube with downward slanted drilled holes for the insects to enter, was used for the upper probe body of the electronic probe (Fig. 1). By using this piece upside down with the insect capture holes slanted upward, gravity tends to keep grain particles out while not affecting the entry of insects (Subramanyam et al., 1989). A tubular outer dust sleeve covers the holes during insertion of the probe into the grain to prevent particles from entering the probe, after which the sleeve is pulled up. For the inhouse produced probes, the sleeve was a rectangular piece of clear flexible plastic (0.025 mm thick) clear acetate sheeting (P/N No. 44008, United States Plastics, Lima, OH) that was rolled to fit the outside of the trap body and secured with electrician's tape along the seam and around the periphery of the top and bottom of the sleeve. For the manufactured probes, the sleeve was a 12 cm section of tenite butyrate tubing (No. 42127, United States Plastics, Lima, OH) with a strip of velcro tape (1 cm wide, hook side) attached to the inside periphery of the top and bottom of the sleeve. The velcro wiper serves to fill the gap between the inside of the tubing and the outside of the probe trap body, and allows the sleeve to slide up the trap after the trap is placed in grain. The lower probe tip and the receptacle were from the Storgard WB Probe II probe trap, which were the same as those used for the first generation prototype.

The sensor head or main probe body (Fig. 1) contained a funnel placed above the beam to direct falling insects through the path of the infrared beam and a funnel placed below the beam to prevent insects from returning to the vicinity of the beam after they have fallen

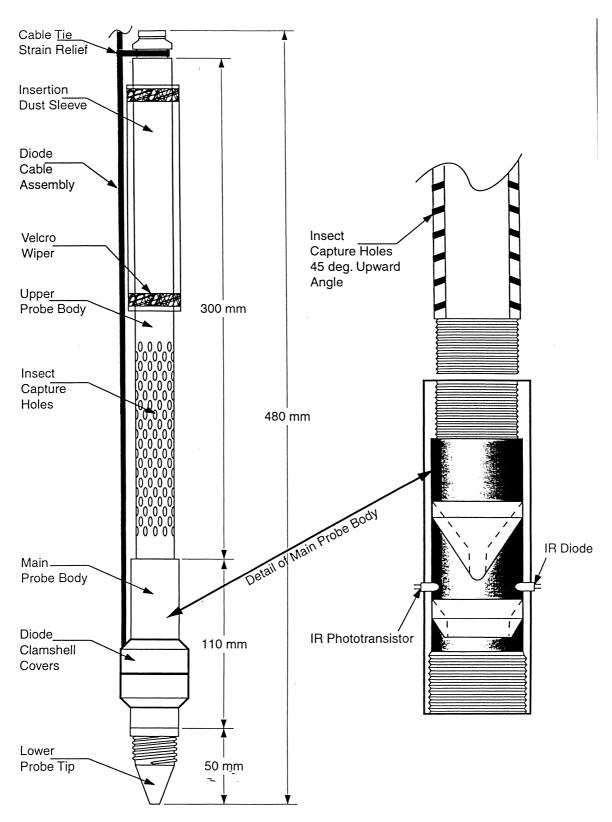


Fig. 1. A diagram of the manufactured prototype electronic probe with enlarged details of the main probe body showing the second generation infrared beam sensor head. The recessed infrared beam transducers and the steep angles of the upper and lower funnels prevent captured insects from remaining in the path of the infrared beam.

through it. The second-generation sensor head was modified by recessing the infrared-beam transducers (IR diode, Lytron 351-1151 and IR phototransistor, Lytron 351-1152; Mouser Electronics, Mansfield, TX) back from the path of the falling insects. Several design features help keep insects from remaining in the infrared-beam and producing erroneous insect counts. The surface of the top funnel was sufficiently steep and was coated with a thin layer of Teflon (polytetrafluoroethylene) applied as a liquid so that insects landing on it continue to fall through. The aperture at the bottom of this funnel was smaller than the beam width to direct the falling insect through the beam. The bottom edge of the funnel was positioned with a gap above the top of the beam so that if any insect inadvertently managed to hang from the bottom of the funnel, it would not intersect the beam and cause multiple insect counts. The sharp acute angle of the bottom edge also helped keep such insects from crawling around and up the outside surface of the funnel (and then down to the infrared-beam transducers). The function of the lower funnel (also with a sharp acute angle bottom edge) similarly was to help prevent insects from crawling up from the bottom of the sensor head to the infrared-beam transducers. The lower funnel's bottom aperture was wide enough so that insects falling through the top funnel passed through without contacting it. When used with the receptacle in laboratory tests with live insects or in field tests, the receptacle was also coated with a thin layer of Teflon to further prevent movement of the insects back up into the sensor head.

In the in-house produced prototype, the body of the sensor head and the funnels were fabricated by machining PVC stock. Manufactured prototypes were produced by Analytical Research Systems (Gainesville, FL). Because of its superior machinability, Delrin plastic (DuPont, Wilmington, DE) was used for production of the body of the sensor head and the funnels in the manufactured prototypes. Black Delrin was used for the body and white Delrin was used for the funnels. White funnels improved ability to visually examine the interior of the sensor head. In preliminary testing, it was determined that the reflectivity of the smooth interior surface of the Delrin sensor head—body interfered with the performance of the infrared beam, which decreased the accuracy of the manufactured probe. This was remedied by abrading the interior surface between the upper and lower funnel with 80 grit carborundum silica carbide paper, using nonuniform two-directional movements.

Two other modifications to the probe cables, incorporated after the FL field test, were employed in all of the prototype probes used in this study. The high impedance sensor leads, used to connect the beam generation/detection circuitry to the sensor head (Shuman et al., 1996), were originally implemented with a two wire (LED and phototransistor signals) cable with a foil shield (also providing a common ground path). Mechanical agitation of these cables could induce small electrical transients, which produced false counts (Arbogast et al., 2000). This problem was eliminated by selection of a three-wire cable with a braided (mechanically stronger) shield (Cable No. 83553, Belden Wire and Cable, Richmond, IN) to separate the common ground current path (one of the wires) and the shielding (since the shield was now only connected to ground on one end). Phosphine fumigation, which was conducted by the bin owner during a previous field test, caused corrosion of the cable connectors in the bin and subsequent system failure (Arbogast et al., 2000). Therefore, sealed, corrosion resistant, Mini-Con-X connectors (Conxall, Villa Park, IL) were used on the cables. A silicon-based material was added in the sensor heads to pot exposed transducer leads (i.e., embed the transducer leads in a protective material).

In addition to the electronic probes, the detection/interface circuit boxes were also produced by Analytical Research Systems, (Gainesville, FL) for use with the electronic probes. A complete description of these circuit boxes has been given previously (Shuman et al., 1996).

2.2. Laboratory tests

Insects used in this study included adults of *Cryptolestes ferrugineus* (Stephens), the rusty grain beetle; *O. surinamensis* and *T. castaneum*. All insects were obtained from laboratory colonies maintained at the USDA/ARS laboratory in Gainesville, FL. Mustard seeds (*Brassica juncea* (L.) var Florida broadleaf) were also used in some tests. Seeds were measured using an ocular micrometer with a stereoscope at $10\times$, and a set of seeds 1.5-mm in diameter was obtained. These seeds are fairly symmetrical spheres and their diameter (1.5 mm) was similar to the length of *C. pusillus* adult. These seeds are smaller in length but larger in width than *C. ferrugineus* adults used in this study, which averaged 2.0 mm \times 0.7 mm.

Baseline accuracy of the manufactured probes was determined with drop tests using dead C. ferrugineus, O. surinamensis and T. castaneum. These insects approximated the size range of pest insects that would be sampled by these traps in stored grain. Probe traps were placed in a rack that held them in an upright position, the outer dust sleeve was left in the lowered position so that the holes were covered, and a small brush was used to drop insects one at a time from the top center of the probe trap. Previous research found that none of the live insects that were dropped through the sensor head were able to hang on the upper funnel, so results obtained with dead insects are equivalent to results obtained with live insects in drop tests (Shuman et al., 2000; N.D.E. & D.S., unpublished data). Insects used for these tests were killed by freezing, were held at room temperature until the time of testing, and were used within 14 days. Ten sets of ten insects (i.e., 100 per species) were dropped through a probe, and the percentage of insects counted electronically was used to determine probe accuracy. The output signal pulse from the phototransistor generated by a falling insect was captured with an oscilloscope (Fluke 97 Scope meter, Fluke, Everett, WA) configured to give its peak value as a digital readout. The phototransistor produces a pulse whose peak value varies with the fraction of the beam masked. Only output signals greater than a user-adjustable (in the detection/ interface circuit box) threshold setting result in an electronic count. Thus counts due to background noise and grain dust particles are avoided, and setting the threshold to appropriate levels can limit the minimum detectable arthropod size. The threshold was set at 60 mV in our studies. There is also a user-adjustable phototransistor operating-point bias adjustment for each of the eight channels in the detection/interface circuit box, which was set to 1.5 V after a probe was attached to a channel in order to compensate for component and sensor head dimensional tolerances. The oscilloscope was used to differentiate between dropped objects that produced no output signals from those in which produced signals that were too small (i.e., below the threshold) to trigger an electronic count, as well as to quantify the output signals. Tests of output signals were conducted with 30 mustard seeds per probe, and mV output per mustard seed drop was recorded.

2.3. Accuracy and performance of manufactured EGPIC system

Initial tests were conducted with eight probes and one detection/interface circuit box that were produced in-house. Two replicates of drop tests of 100 C. ferrugineus per replicate were conducted to measure average percent accuracy and to determine range of accuracy obtained. This information was used to determine performance specifications for the manufactured prototypes. After producing a model manufactured prototype probe that met performance specifications, an additional 54 prototype probes and five circuit boxes were produced by the small-scale manufacturing process. The five circuit boxes were tested with drop tests of dead insects only using the model manufactured prototype probe on all eight channels of all five boxes. Data were subjected to the Box–Cox procedure, which is a power transformation that regresses log-transformed standard deviations (y + 1) against log-transformed means (x + 1) (Box et al., 1978), and data were transformed to stabilize the variance before analysis when necessary. One-way analysis of variance (ANOVA) within each insect species using Proc GLM (SAS Institute, 1985), followed by LSD mean separation test (P = 0.05) for significant ANOVAs, was used to compare accuracies of the circuit boxes.

The 54 manufactured probes were then tested on the five manufactured detection/interface circuit boxes (with 8–16 probes tested per box) and compared with the eight in-house probes tested on the in-house circuit box. Two sample *t*-tests using Proc TTEST (SAS Institute, 1985) were used to compare in-house and manufactured prototype probes. All probes were tested with drop tests of 100 insects each of *C. ferrugineus*, *O. surinamensis* and *T. castaneum*; and with drop tests and oscilloscope measurements on 30 mustard seeds. Parameters recorded included accuracy for each insect species and for mustard seeds, and average and standard deviation of output signals from mustard seed drops per probe. Correlations among these parameters were determined using Proc CORR (SAS Institute, 1985).

3. Results and discussion

Accuracy of the in-house produced prototype probes averaged (\pm standard deviation) 90.9% (\pm 3.58) and ranged from 84% to 97% in individual probes in tests with dead *C. ferrugineus* in preliminary tests using an in-house produced detection/interface circuit box. Therefore, production specifications for the manufactured prototype probes were set at \geq 85% accuracy for *C. ferrugineus*. A model manufactured prototype probe was produced and tested on all channels with both an in-house produced detection/interface circuit box and four manufactured circuit boxes. There were no differences in accuracy between the in-house produced and the manufactured circuit boxes with *O. surinamensis* (F = 0.44; df = 4,35; P = 0.7787) and *T. castaneum* (F = 1.00; df = 4,35; P = 0.4207), and average accuracy ranged from 97.0% to 97.9% and 99.3% to 99.8%, respectively. There were differences in accuracies for counting *C. ferrugineus* among the circuit boxes (F = 2.67; df = 4,35; P = 0.0484). The in-house produced box was less accurate than two of the manufactured boxes, but there were no differences among the manufactured boxes.

Tests were then conducted that compared 54 manufactured probes with eight in-house probes. Accuracy of the in-house probes tended to be higher than that of the manufactured

probes for O. surinamensis, T. castaneum, and mustard seeds, and was significantly higher for C. ferrugineus (Table 1). Accuracy of individual probes for counts of C. ferrugineus ranged from 92% to 100% for the in-house probes and from 87% to 100% for the manufactured probes. There tended to be larger output signals produced by mustard seeds dropped through the in-house probes and the standard deviation of the output signals tended to be greater in the manufactured probes (Table 1). Correlation analysis was used to evaluate the relationship among all of these parameters (n = 62). The accuracy in counting mustard seeds of 1.5 mm diameter was positively correlated with accuracies for counting T. castaneum (r = 0.48901, P = 0.0001), O. surinamensis (r = 0.33073, P = 0.0087), C. ferrugineus (r = 0.33442, P = 0.0079), and negatively correlated with output signal standard deviation (r = -0.39033, P = 0.0017). The accuracy in counting C. ferrugineus was positively correlated with accuracies for counting T. castaneum (r = 0.27318, P = 0.0317) and O. surinamensis (r = 0.30798,P = 0.0149), and negatively correlated with output signal standard deviation (r = -0.56542, P = 0.0001). Finally, there was a positive correlation between output signal average and standard deviation (r = 0.25445, P = 0.0460). There were no correlations among the other parameters.

Good performance was obtained with the manufactured prototype probes. The accuracy of the probes increased as the size of the insect increased. The larger insects consistently pass through a large enough area of the beam to produce an output signal greater than the threshold level. When problems were encountered with accuracy of a manufactured probe, it was remedied by replacing the diode and phototransistor. Initially, it was thought that inadequate diode irradiance output and/or phototransistor sensitivity (as indicated by the size of the output signal) was the primary factor responsible for inaccurate insect counts. However, our study found that probe accuracy was correlated with variation in output signal. The infrared beam intensity is not uniform throughout its cross-section, possibly due to imperfections or variations in the lens of the diode and/or phototransistor. The insect outline is irregular and orientation of the insect as it passes through the infrared beam will affect the magnitude of the output signal. An insect that presents a small profile as it falls through the

Table 1 Mean \pm standard deviation obtained in drop tests using recently killed insects, 100 per probe per species and 1.5 mm mustard seeds, 30 per probe. n = 54 manufactured, n = 8 in-house probes; df = 60

	Prototype tested		t	P
	In-house	Manufactured		
Tests with insects:				_
C. ferrugineus accuracy (%)	96.5 ± 2.98	93.6 ± 3.63	2.1684	0.0341
O. surinamensis accuracy (%)	99.3 ± 0.88	98.3 ± 1.48	1.7372	0.0875
T. castaneum accuracy (%)	100.0 ± 0.00	99.5 ± 0.69	1.95052	0.0558
Tests with mustard seeds:				
Accuracy (%)	99.3 + 1.39	97.0 + 3.52	1.7462	0.0859
Output signal average (mV)	$\frac{-}{561.6 + 66.67}$	519.1 + 71.94	1.5655	0.1227
Output signal standard deviation (mV)	165.7 ± 26.99	192.1 ± 45.30	1.5946	0.1161

beam and that passes through a weak section of the beam will produce a much smaller signal than the same insect that presents a large profile (Fig. 2). Some of these variations were removed by using the spherical mustard seeds. Even though the seeds were smaller than *C. ferrugineus*, accuracy in counting the seeds was higher. Variation due to irregularity in insect profile can not be reduced. However, use of diodes with a more consistent beam or modifications to improve the focus of the beam may further improve accuracy.

Drop tests with dead insects give a measure of optimal probe performance. However, accuracy with live insects and tests under field conditions are needed to further evaluate system performance. For example, dockage particles falling through the sensor head could generate false overcounts and insects dropping through the sensor head while clinging together would result in undercounts (Arbogast et al., 2000). Additional research is also needed to guide management decisions based on trap capture information, whether the counts are obtained from visual inspection or electronically. Electronic counts, however, will provide information





Fig. 2. Stop-action photographs of live *T. castaneum* falling through the (invisible) infrared beam between two funnels in the sensor head of an electronic probe. Depending on the position when the insect crosses the beam, a small profile (left) or large profile (right) may be presented. The original photographs were taken in a dark room, and the passage of the insect through the infrared beam, which triggers an electronic count of the captured insect, was used to trigger the camera.

on periodicity of insect capture within a day or changes in rate of insect capture over time, which is not available when traps are checked only weekly or biweekly, and this may also aid in making management decisions.

For initial field evaluation, the trap receptacle would remain in place, so that probes could be removed and the actual number of insects captured could be compared with the number of electronic counts. However, for standard deployment, the receptacle could be replaced with a release mechanism that would allow the insects to be released at a point away from the probe trap so that the insects would not reenter the probe trap and be counted multiple times. Probes would need to be removed periodically to be cleaned and maintained, and the frequency of this activity would depend on the condition of the grain. However, since the probes would not need to be removed to determine the number of insects captured, there would be a reduced need to reenter the grain bin. This would increase worker safety and provide information that is currently unavailable for management decisions.

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